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Bacterial colonisation of fresh and dried perennial ryegrass in the rumen

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Introduction

The first step of degradation of plant material within the rumen involves rapid colonisation of the material by a complex bacterial community¹. Previously, colonisation of conserved hay stems by cellulolytic bacteria (*Fibrobacter succinogenes* (Fs), *Ruminococcus albus* (Ra) and *R. flavefaciens* (Rf)) was shown to occur at equal rates². However, how this compares to fresh grass is unclear.

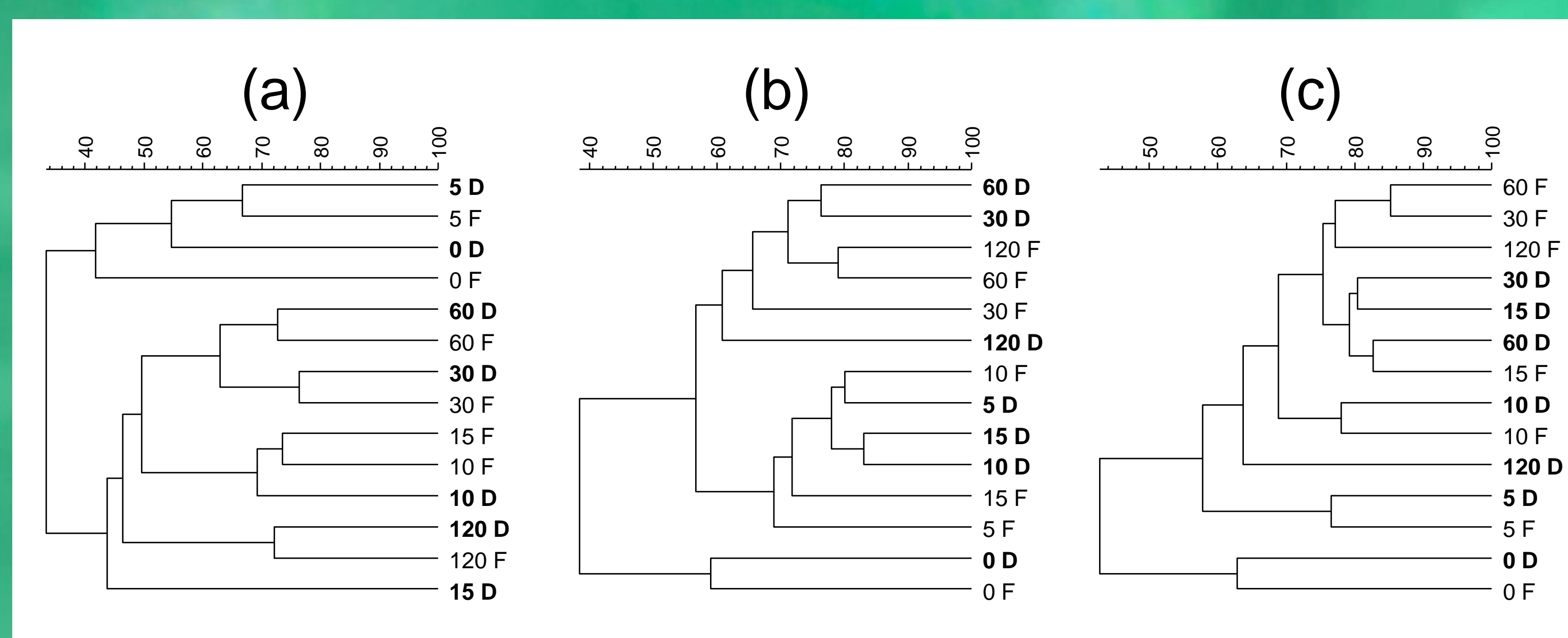
Aim

To characterise early (<2 h) populations of rumen eubacteria and cellulolytic bacteria colonising fresh and dried perennial ryegrass (PRG), and determine the corresponding dry matter (DM) loss.

Method

- Fresh or dried PRG (mechanically processed to mimic mastication) was incubated *in sacco* in the rumen of three rumen fistulated, non-lactating dairy cows grazing a ryegrass sward.
- For each cow, duplicate polyester bags of each forage type were incubated per time point (5, 10, 15, 30, 60 and 120 min) with 0 min bags processed directly. Bag residues were hand washed and snap-frozen in liquid N. Rumen contents were also sampled (0, 60 and 120 min) and snap-frozen.
- DNA was extracted from the residual DM (RDM), and the colonising bacteria analysed by eubacterial 16S ribosomal DNA based denaturing gradient gel electrophoresis (DGGE)¹ and quantitative PCR (eubacteria¹, Fs², Ra² and Rf²). Rumen contents were analysed similarly.

Fig 1. Cluster analysis (% similarity) of DGGE profiles of the eubacteria colonising fresh (F) and dried (D) PRG incubated in the rumen of three different cows (a-c). Branch labels denote incubation time (min) and PRG preparation (e.g. 120 F).



Results

- PRG preparation and incubation time did not affect the composition of the colonising rumen eubacterial populations consistently (Fig 1).
- Colonising eubacteria, Fs, Ra and Rf increased over time ($P < 0.01$), and were greater with fresh PRG than dried ($P < 0.001$) (Fig 2).
- Relative abundance of the cellulolytic bacteria in rumen content was $Rf > Fs > Ra$ for all cows, but for colonising cellulolytic bacteria the relative species abundance differed by cow (data not shown).
- Initial DM loss (0 min) was greater with dried PRG than with fresh (18.4 v 5.5 %; $P < 0.01$).
- A linear interaction ($P < 0.001$) between forage and time in terms of DM loss (relative to 0 min) was observed (Fig 3), with dried PRG showing greater apparent DM loss after 2 h than fresh PRG (25.0 v 7.3 %; $P < 0.05$).

Fig 2. Eubacterial DNA (a) and cellulolytic bacterial DNA (b-d) on fresh (red) and dried (black) PRG incubated *in sacco* in the rumen. Data points represent the mean of duplicate bags for each cow.

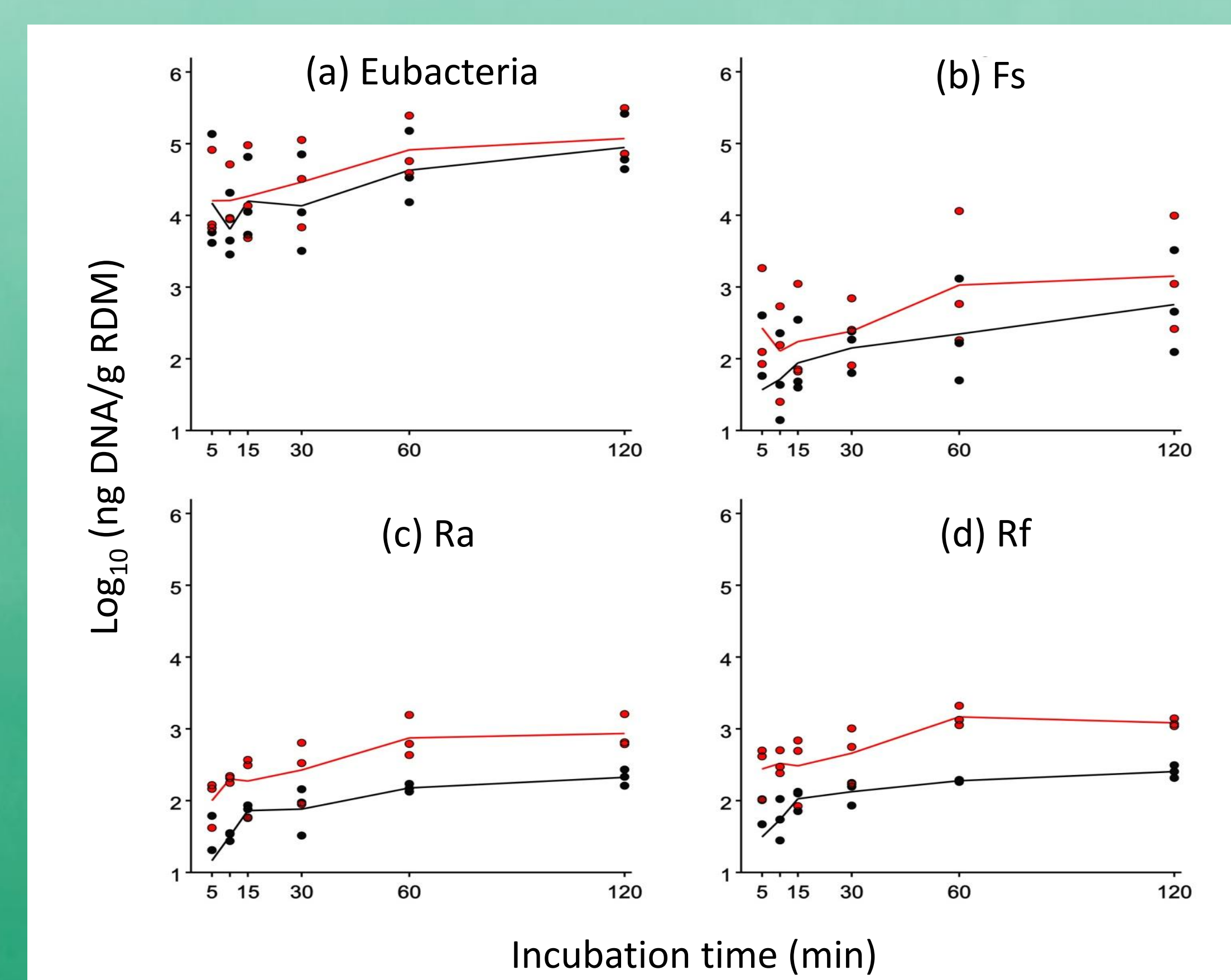
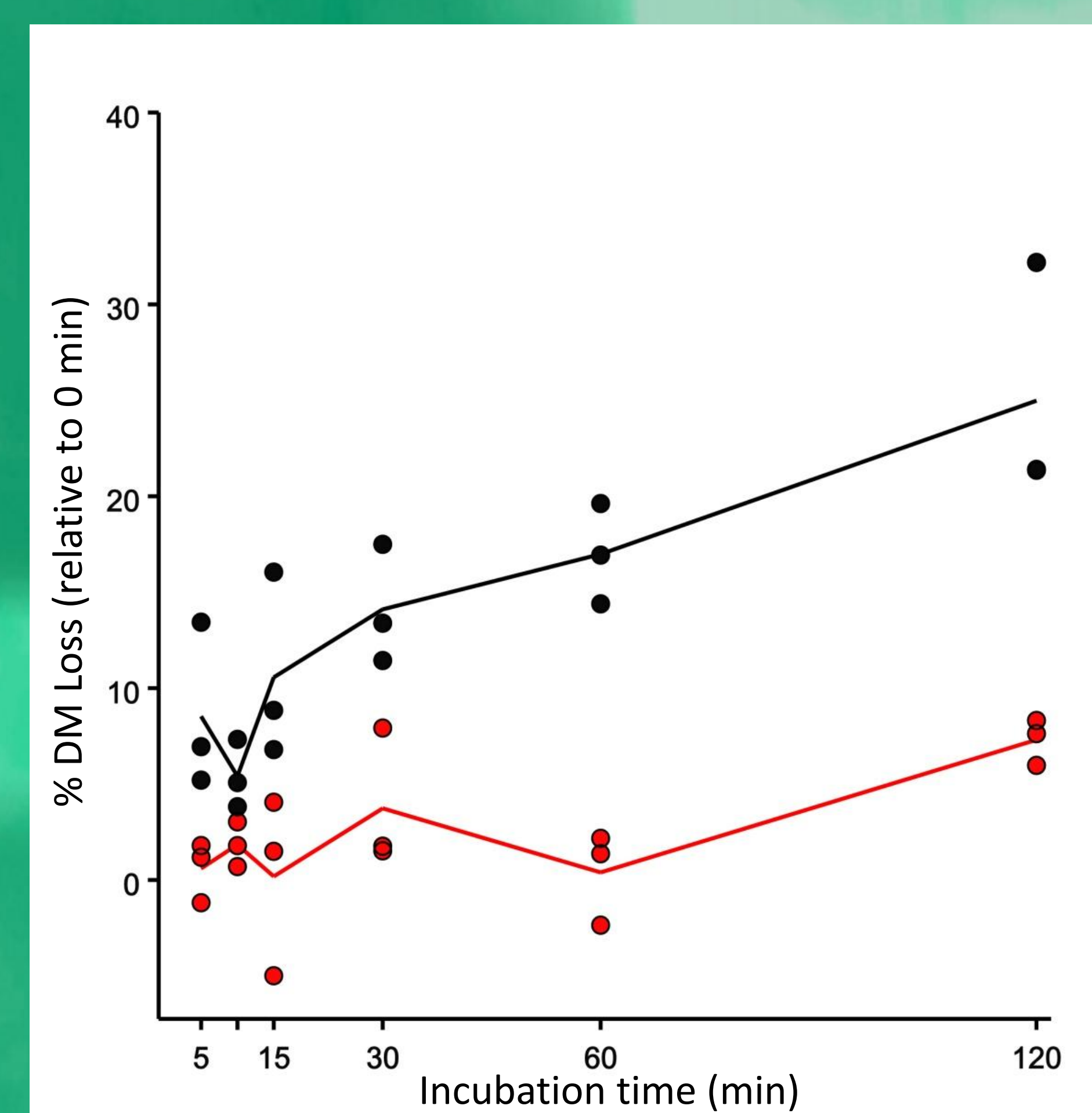


Fig 3. DM loss from fresh (red) and dried (black) PRG incubated *in sacco* in the rumen. Data points represent the mean of duplicate bags for each cow.



Conclusion

- Colonising rumen bacterial populations were larger with fresh rather than dried PRG, but this was not reflected in DM loss.
- Animal differences in relative abundances of colonising cellulolytic bacteria were more apparent than any forage associated effect on total population composition.
- Clarification as to whether the observed differences in initial and ruminal DM loss may have resulted from differing responses to the mechanical processing (to mimic mastication) is required.

References

- Edwards JE, Huws SA, Kim EJ & Kingston-Smith AH (2007) *FEMS Microbiol. Ecol.* 62, 323-335.
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